University of Nevada, Reno

Transitions: The Search for Maize Phytoliths in Spanish Teeth

A thesis submitted in partial fulfillment of the
requirements for the degree of

Bachelor of Arts in Anthropology and the Honors Program

by

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May 2010

UNIVERSITY OF NEVADA RENO

THE HONORS PROGRAM

We recommend that this thesis
prepared under our supervision by

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entitled

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be accepted in partial fulfillment of the
requirements for the degree of

Bachelor of Arts in Anthropology

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Abstract

This research aims to determine if plant phytoliths can be recovered from human dental calculus. This paper hypothesizes that phytoliths will provide a more direct method for paleodietary analysis than carbon and nitrogen isotopes. If this method is successful, researchers in bioarchaeology will then be able to identify directly the types of plants ingested by earlier human populations. In this blind experiment, dental calculus from the teeth of pre- and post-AD 1500 Spanish skeletons are subjected to a serial dilution in an acid bath to remove phosphorous, calcium, and any organic residual matter. The final dilutions are centrifuged to separate and isolate the phytoliths. The resulting precipitates are placed on slides and analyzed under a darkfield microscope (400 – 1600X). Potential phytoliths are then photographed and compared to enlarged images of known phytoliths.

Acknowledgements

I would like to express my gratitude to my mentor, Dr. G. Richard Scott, for gathering the dental calculus, endlessly proofreading my paper, teaching me how to be a better writer, and giving me the guidance which enabled me to develop a deep understanding of the subject matter.

I would also like to thank Dr. Tamara Valentine for proofreading my paper and catching mistakes I would have otherwise missed. You pushed all of this year's Honors graduates to do the best we could and for that I thank you.

I would also like to thank the HURA Committee for funding this project. Without this money, this experiment would have never been completed.

I would also like to thank Kristy Peck who showed patience and understanding while helping me complete the Chemistry part of this experiment. You are truly the best Chemistry teaching assistant I have ever had.

Lastly, to all the people who supported me in this endeavor, I offer a most sincere thank you.

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Introduction

While plants are made up primarily of organic molecules, notably carbohydrates and proteins, they also include an inorganic fraction taking the form of phytoliths. Following the death of a plant, the organic fraction typically disappears entirely, through oxidation and the action of detrivores, leaving the phytoliths in the surrounding soil. Because of this abundance in soil, phytoliths are ubiquitous in the archaeological record. In fact, a library or database search of the word *phytolith* produces numerous results detailing their relevance in archaeology and paleoecology. Rarely, though, is their importance in physical anthropology reported. The reason is that the only place phytoliths are found in the human body is in dental calculus. While the potential for analyzing phytoliths in dental calculus is enormous, the reason they have not been more fully exploited to reconstruct paleodiet is that the methods involved in extraction, isolation, and identification are complex, time-consuming, and expensive.

When bioarchaeologists analyze paleodiets, they focus primarily on the stable isotopes of carbon and nitrogen that are incorporated into human bone during the lifetime of an organism. However, carbon and nitrogen isotope analysis is only an indirect proxy of diet as it does not tell exactly what was consumed. For example, tropical grasses, such as maize, are C_4 plants while the C_3 isotope characterizes temperate grasses. In skeletal remains, an increased C_4 signature strongly suggests the introduction of maize in xeric (extremely dry) habitats, which usually lack C_4 plants. I hypothesize the presence of phytoliths in dental calculus is not a proxy indicator but provides direct evidence of what an individual consumed. To test this theory, I will perform an experiment searching for maize phytoliths in post-Medieval Spanish teeth.

Terms to Know

At the outset, it is necessary to define the terms *phytolith*, *calculus*, and *maize*. Phytoliths go by many different names: silica phytoliths, opal phytoliths, biogenic opal, plant opal, and silica cells. To limit confusion, the word *phytoliths* is used as an umbrella term for all those listed above. No matter which term is used, they all refer to the same thing: "particles of hydrated silica formed in living plants that are liberated from plants after they die and decay" (Piperno, 2006:1). Essentially, phytoliths help form the structure of the plant's cell walls, but not all plant species produce phytoliths. For example, Nymphaeaceae (water lilies), Agavaceae (agaves), and Caricaceae (papaya) are all families of plants where phytoliths are either rare or completely absent. Cyatheaceae (tree ferns), Poaceae (grasses – to include maize), and Ulmaceae (elms), on the other hand, are families of plants that produce high amounts of phytoliths. For more examples, see Fig. 1 (Appendix A). Many different factors contribute to whether or not a plant produces phytoliths: climate, soil type, amount of water in the soil, the age of the plant, and the plant's taxonomic affinity. Relative to these factors, phytolith analysts are better able to predict which genera and families will have phytoliths and those where they will be rare or absent. Of the plants that develop phytoliths, each has its own unique type, thus acting as a plant's fingerprint. For example, the phytoliths of the seeds in the family Marantaceae are long with smooth, conical ends while circular, scalloped phytoliths are found in the fruit rinds of the family Cucurbitaceae (Figs. 2 and 3).

Why plants produce phytoliths, and whether it is beneficial for the plants to expend energy to do so, is subject to debate. While some researchers (Kaufman et al., 1985) argue silica cannot be considered essential because its role in metabolism of grasses is not known, others (Epstein, 1994) contest an element should not be classified as essential just because it confers advantages – growth and reproductive success – in certain environments. One could also argue if phytoliths are an essential component in a plant's survival, all plants should have them. This is not the case.

Regardless of whether or not phytoliths are beneficial to plants, they have been the focus of extensive research. Piperno (2006) outlines four important stages of phytolith research that are briefly outlined here.

1. *Discovery and Exploratory Stage (1835-1895):* The German scientist Ehrenberg observed siliceous bodies in soil samples and named them Phytolitharia (Greek for *plant stone*). He then developed the first phytolith classification system.
2. *Botanical Phase of Research (1895-1936):* Archaeological applications were used for phytolith analysis and several researchers identified phytoliths in grasses (e.g., barley, millet, and wheat).
3. *Ecological Research Stage (1955-1975):* A classification system permitting the distinction of three subfamilies of grasses was developed by Twiss et al. (1969). The United States, Britain, and Australia began conducting phytolith research at this time. Previously, only Germany had been doing so.
4. *Modern Period of Archaeological and Paleoenvironmental Research (1978-):* This is the era when researchers confirmed phytoliths occur in diverse shapes, can be direct proxies of human diet and plant

> exploitation, and are plentiful in late Quaternary archaeological and paleoecological sites.

Calculus is important to understand because it is the only substance in the human body that can incorporate phytoliths. More commonly referred to as tartar, calculus is made up of several minerals, including brushite, octacalcium phosphate, whitlockite, and hydroxyapatite where the primary elements are phosphorus, calcium, and magnesium. Typically, a layer of active plaque covers calculus. When individuals consume vegetable foodstuffs, the phytoliths in food can be incorporated into plaque and permanently preserved in calculus. For example, Armitage (1975) found festucoid grass phytoliths in cattle calculus from prehistoric, Roman, and medieval sites in Britain. Hillson (1986:302) avers "there is potential for recovery of a wide variety of plant and other food remains from calculus."

Calculus takes different forms in different animals. It can be chalky in horses and hard and stained in dogs. In humans, supragingival (above the gumline) calculus has a hard, clay-like consistency and is firmly attached to the tooth. Subgingival (below the gumline) calculus has a thinner deposit, is harder, more heavily mineralized, stains a dark brown to green-black, and adheres to the teeth more firmly than supragingival calculus. Of the two, supragingival calculus is more common, although subgingival deposits increase with age. In humans, supragingival calculus typically deposits on the lower incisors and upper molars. For examples of what dental calculus in skeletons look like, see Figs. 4, 5, and 6.

The amount of calculus buildup varies throughout the mouth. This could occur for many reasons: salivary flow rate, plaque accumulation, phosphate and calcium levels in the blood, and mineral content in water. Interestingly, in an experiment where the salivary glands of rats were removed, calculus did not form (Baer et al., 1963). Lieverse et al. (1999:332) also found “calculus deposition is facilitated by an alkaline oral environment and that diets high in protein increase oral alkalinity.”

Balsas teosinte is the wild ancestor of maize (*Zea mays*). Because maize phytoliths have been found in a variety of archaeological and paleoecological contexts, researchers suggest maize was domesticated and dispersed out of Mexico by the early seventh millennium B.P. Piperno (2006) notes that this finding parallels a molecular clock showing that maize domestication occurred around 8000 B.P.

Unlike the domestication of other cereals, maize’s creation was complicated because its derivation from teosinte involved changes to the plant’s cob structure, architecture, and kernel and row numbers. A potential avenue of research for archaeologists or paleoecologists would be to see if the maize phytolith shape can tell researchers how it was domesticated (i.e. what other plant it was crossed with). There are several theories as to how maize became domesticated:

1. Smaller domesticated maize could have been crossed with either *Z. luxurians* or *Z. diploperennis* of the teosinte section *Luxuriantes* (Ordish et al., 1996).
2. It could have evolved from a hybridized version of *Z. diploperennis* by a species of Tripsacum. This is not a widely accepted theory (Ordish et al., 1996).
3. It could have been directly domesticated from teosinte (Ordish et al., 1996).
4. There might have been two or more domestications of teosinte (Ordish et al., 1996).

There are always going to be conflicting theories on how maize was domesticated, but no matter the cause, it is undeniable that maize is the most famous and widespread crop in the world. It is, and will continue to be, the staple food of many societies. Importantly, it also produces several different forms of phytoliths that are clearly associated with the maize plant.

Literature Review

To put this project in context, journal articles and books provide information on what has been accomplished to date using phytoliths in paleodietary and paleoenvironmental reconstructions. Following are brief summaries of research that were most informative and helpful during this research.

Fox et al. (1996) conduct research that parallels closely the maize phytolith experiment. Rather than attempting to establish associations between microwear patterns in teeth to specific foods, Fox et al. (1996) try to understand the causes and mechanisms of microwear formation. In addition to this, they attempt to recover phytoliths from human dental calculus using different methods than those used by previous researchers. First, the teeth of seven individuals, with medium amounts of dental calculus, are collected from a Late Roman necropolis in Tarragona, Spain. To remove potential contaminants to the phytoliths, the teeth are cleaned with a toothbrush and distilled water. The researchers take small fragments of calculus from each individual, put them on stubs, crush them with tweezers, and cover them with gold. They take more calculus fragments, submerge them in a 20% HCl acid bath for six to twelve hours, place the resulting residue on a stub, and coat it with gold. Following this treatment, Fox et al. (1996) note that phytoliths are resistant to the effects of acid and the resulting residue is primarily phytoliths. One basic question during this experiment is whether or not the phytoliths in the calculus would be destroyed if they are left too long in an HCl acid bath. The results from Fox et al. (1996) suggest that overexposure to acid is not a problem in phytoliths preservation.

For comparative purposes, Fox et al. (1996) examine enamel surfaces and burial soil for phytoliths. For the enamel surfaces, they extract one tooth from each individual and coat it with gold. For the burial soil, they assume phytoliths from the stomach of the decaying corpses would be deposited around the lumbar vertebrae and the sacrum, so they gather soil deposits from this area. In each comparative case, a scanning electron microscope (SEM) is used. When the dental calculus and enamel are viewed, the magnification is at 400X. If phytoliths are found, the magnification is increased to 3,000-5,000X. Three-dimensional images are then obtained, photographed (see Fig. 7), and classified by comparing their micrographs with a reference sample of present-day plants from the Mediterranean.

Since the methodology Fox et al. (1996) utilize to obtain phytoliths from dental calculus is somewhat analogous to the methodology I utilize in the maize phytolith experiment, only its results will be discussed. Fox et al. (1996) find numerous phytoliths embedded in the calculus. The identification of some phytoliths is hindered because substantial carbonate deposits cover them. Phytoliths are also found in the samples treated with HCl, but these are mixed with some undigested calculus which appear similar to silica phytoliths. X-ray microanalysis is used to identify the real phytoliths given their siliceous composition (see Fig. 8). In my maize phytolith experiment, some undigested Spanish calculus also did not fully dissolve in the HCl acid bath. Because X-ray microanalysis could not be used to distinguish between real and fake phytoliths in my experiment, it was harder to determine what was a phytolith and what was calculus.

Fox et al. (1996) conclude their study by saying that phytolith research, while useful in many areas, is most promising for reconstructing the diets of past human populations. They appear confident, despite limitations in the study, that the potential to shed light on past human diets is ready to be exploited, but researchers need to expand their knowledge of modern phytoliths.

Although her work is aimed primarily at archaeologists and paleoecologists, Piperno (2006) explains what phytoliths are, how they are made, and the many different taxonomies associated with them. Explained in great detail in this book is how and why plants make phytoliths. There are two mechanisms plants use to produce phytoliths. The first mechanism is controlled by plants and, through genetic and physiological processes, phytoliths are formed in cells and tissues. This mechanism is designated for the accumulation of solid silica. The second mechanism is much more passive than the first: it is dependent on the climate and growing conditions of the plant. Therefore, the outcome is random. Interestingly, in many plant species, both mechanisms can be at work in different areas of the same plant. Much research is still needed as researchers are not well informed about the specific metabolic pathways involved in the formation of phytoliths.

Why plants make phytoliths is still relatively unknown and many researchers debate how essential they are to plant form and function. Some researchers (Sangster et al., 2001) have characterized the major functions of phytoliths as structural, physiological, and protective. Piperno (2006) posits a plant must have a good reason for using its energy to accumulate silica from soil. She notes that it is not a successful strategy for any organism to waste energy and get nothing in return. In some plants (i.e. horsetails, rice, and beets), silica is essential for normal growth and development. Yet in others, silica is not a necessary element so they do not waste metabolic resources to obtain it.

Piperno (2006) describes in detail the morphological differences between teosinte phytoliths and maize phytoliths as the former is often confused with the latter. Teosinte has a rectangular to trapezoidal shape, sometimes decorated with small knobs or protuberances. It can also take a rondel shape with two circular faces, similar in size, and connected by a plate of silica (see Fig. 9). Phytoliths characteristic of teosinte are either rare or not present in maize, making identification of one or the other relatively simple. When maize phytoliths are rondel shaped, they can be distinguished from teosinte phytoliths because they are not often decorated (as teosinte is). When they are decorated, they do not possess the undulating margins of teosinte phytoliths. Instead, they are characterized by either edges with a sawtooth pattern or plain jagged edges. The face of the maize phytolith is also much smaller than the face of the teosinte phytolith (see Fig. 10).

Rovner (1988) outlines several reasons why phytolith studies have been rare or significantly underdeveloped. One reason is because the taxonomy of phytoliths is so complex. That is, the forms they take can be staggering and even the same species of plant can have many different forms (maize, for example, has about four different phytolith shapes). Rovner asserts this is not as much of a problem today as it once was since plant groups can now be identified using phytolith populations and diagnostic phytolith forms at the family, subfamily, and genus levels. The second reason is environmental factors that can move soil (water, wind, etc.) can move phytoliths too. Rovner (1988:156) classifies this reason as a complete myth, stating “phytolith content in a stable soil horizon stays in that horizon.” This thesis is intent on showing physical anthropologists that working with phytoliths is not only possible, but also a valuable resource to exploit. Because of these reasons, though, scientists are less inclined to even try to understand the benefits of using phytoliths.

Relative to my maize phytolith experiment, Rovner (1988) asserts phytolith analysis is the best microfossil system for studying cultivated cereals, particularly in the study of maize. Like Fox et al. (1996), Rovner (1988) avers that before the potential of phytoliths can be successfully tapped, time and resources must be dedicated to developing a reference database detailing phytolith systematics.

Lu et al. (2002) list the different shapes phytoliths take depending on a plant’s environment. Phytoliths from warm, moist climates produce bilobate (dumbbell) and cross-shaped phytoliths while those representative of colder climates produce trapezoid and rondel phytoliths. Warm, dry climates produce short-saddle phytoliths while tropical and subtropical humid climates produce long-saddle type phytoliths (see Figs. 11, 12, 13, and 14). This is important because if the phytolith shapes of a plant in a particular region change over time that could indicate what the terrestrial climatic conditions were like at specific moments in history. Another potential avenue of research to explore in the current experiment is to look at the shapes of the maize phytoliths to see what the climate in Spain was at the time the maize was consumed.

Gugel et al. (2001) and Danielson et al. (1998) examine the formation of microwear on human dental enamel and infer what phytoliths could have caused the damage. They test the abrasiveness of phytoliths on tooth enamel and how certain phytolith shapes can leave cereal-specific microwear. Because human population growth is linked to food supply, Gugel et al. (2001) emphasize the importance of reconstructing the paleodiets of past civilizations. Therefore, if people in past civilizations went through drastic physical changes in appearance, the food consumed probably was the main cause. The phytoliths extracted from the dental calculus can provide information on what food was eaten. Like Fox et al. (1996) and Rovner (1988), Gugel et al. (2001) note that phytoliths recovered from the dental calculus of human skeletons could be valuable for reconstructing paleodiets and paleoenvironments since they are generally resistant to decomposition.

To locate the phytoliths in their samples, Gugel et al. (2001) do not use an HCl acid bath, but an Academic Center for Dentistry Amsterdam (ACTA) device. This allows them to put the entire enamel in the device as opposed to scrapping off the dental calculus. From the experiment, they are able to confirm opal phytoliths have abrasive potential (as they are ensnared in the tooth enamel), but they emphasize other debris (quartz and other silica particles) should also be taken into account. Gugel et al. (2001:124) are confident that “[t]his experimental approach is likely to further define ancient human dietary behavior, including food processing.”

Danielson et al. (1998) posits the dental microwear in human skeletons from the lower Pecos region resulted from calcium oxalate crystals. They conclude this because hunter-gatherers of the region were dependent on desert succulent plants that have high concentrations of calcium oxalate phytoliths. The dental problems incurred by the hunter-gatherers were dental caries, dental wear, and antemortem tooth loss. What is most interesting about this article is how the researchers test the hardness of the phytoliths relative to the hardness of the enamel. They use a Moh test. The hardness of tooth enamel is between 4.5 and 5.0, so the phytoliths are tested against tiles of this strength. Danielson et al. (1998) find the calcium oxalate phytoliths to be harder than enamel, as they chaff the tiles, providing support for their hypothesis.

Both Hillson (1986) and Lieverse (1999) provide background knowledge on dental calculus. Hillson (1986) describes in detail what is in calculus depending on what animal it came from. In calculus there are minerals such as apatites, whitlockite, and brushite. In humans, there is also the mineral octacalcium phosphate. Hillson (1986) notes that calculus is made up of between 70% to 90% mineral and 10% to 30% plaque bacteria and matrix. He also points out that sites closest to the ducts of the main salivary glands tend to have calculus form near them. This could be direct evidence the main cause of calculus is salivary factors (which would explain why some people develop more calculus than others).

While Lieverse (1999) goes into detail about what dental calculus is and how it is formed, she goes one step further when she describes its usefulness to anthropologists. For example, anthropologists can analyze dental calculus for the degree and presence of periodontal disease in ancient populations. While this information may not be useful in the context of the present study, it is helpful in understanding the different ways calculus can be used to understand past cultures. Something more closely related to my maize phytolith experiment is when Lieverse (1999:219), while still explaining the importance of dental calculus in anthropology, says the "analysis of food debris incorporated within dental calculus deposits can reveal specific constituents of ancient diets...and the extent of dental calculus formation is used by anthropologists as an indicator of ancient dietary patterns." She points out how researchers fail to correlate changing dental calculus levels with changing dietary patterns of different cultures. With the changing diets of the Spaniards after the introduction of Native American domesticates, there were probably changing dental calculus levels too.

All these journal articles and books provide useful information on phytoliths and calculus. They describe varied methodologies (particularly Fox et al. [1996]) and avenues of research to follow, although some of their ideas fall outside the realm of physical anthropology, with greater applicability to archaeology and paleoecology.

Methods

In 2008, G. Richard Scott and two anthropology graduate students gathered dental calculus from about eighty Spanish skeletons from the Cathedral of Santa Maria in Vitoria, Spain. These remains date before and after the critical date of A.D. 1500, which marks the end of the Medieval Period and ushers in the Age of Discovery. As Spanish conquistadors established a foothold in the Americas, their primary aim was to take Aztec and Inca riches back to Europe. Although secondary at the time, a far more important result was the introduction of Native American domesticates to Europe, including tomatoes, pumpkins, cucumbers, and, most importantly, maize (Crosby, 2004). Rather than sorting through the hundreds of phytoliths potentially incorporated into Spanish calculus, the focus of this experiment was centered on the identification of maize phytoliths. This most important staple in the New World became a fundamental crop in Spain after A.D. 1500.

Not all the dental calculus collected from the eighty Spanish skeletons was utilized in this experiment. To make the experiment more manageable, only seventeen samples were used. Because the skeletons were excavated from different, numbered areas of the Cathedral (see Fig. 15), the dental calculus samples were assigned corresponding numbers. All tests were done blind regarding sample age to help make certain false positives were not identified in pre-Medieval calculus.

The methodology I used in this experiment was developed by Dennis Cash and Savanna Schuermann, former UNR students, who also attempted to extract phytoliths from dental calculus. The first step in extracting maize phytoliths from dental calculus was to subject the calculus to a serial dilution in an acid bath (32% HCl) which removed clays, carbonates, and organic materials. To do this, I separately crushed dental calculus (approximately 1 μm in size) from each of the following samples in a crucible to a fine powder.

Sample Number	Age	Sex
15-20	21-35	Male
17-53	>50	Probable Male
17-68	40-50	Unknown
17-70	>30	Unknown
17-77	>40	Male
17-86	35-45	Male
17-103	25-35	Probable Male
19-7	21-35	Probable Female
19-15	21-35	Male
19-17	30-40	Female
19-23	21-35	Probable Male
29-4	36-55	Probable Female

29-27	21-35	Male
29-48	21-35	Probable Male
29-67	21-35	Male
29-159	36-55	Probable Male
62-71	Unknown	Unknown

Then I placed each sample into a separately labeled centrifuge tube and filled the tubes with 8 mL 32% HCl. After letting the samples sit for four days to a week in this acid bath, the final dilutions were centrifuged for five minutes at 3000 rpm (rotations per minute) to separate and isolate the phytoliths. This created a precipitate which contained only the siliceous phytoliths. At the end of centrifuging a liquid, there were two separate layers. I pipetted out the top ¾ layer, discarded it, and replaced it with double distilled water (2xH2O). Again, the dilutions were centrifuged for five minutes at 3000 rpm. Finally, I prepared several slides (three for every one sample) from each precipitate to maximize the probability of finding maize phytoliths.

Following slide preparation, a darkfield microscope (400-1600X) was used to search each slide for the presence of phytoliths. Upon finding a phytolith, the goal was to take a photograph of it with a microscope camera. The image would be pulled up on a computer, enlarged for better viewing, and compared to known images of different plant phytoliths (maize, potatoes, squash, beans, etc.).

Results

Potential maize phytoliths were successfully found in Spanish sample 15-20. Unfortunately, despite these findings, failures in the software interface between the microscope and computer precluded digital photos of my findings. I instead drew pictures of their morphologies and noted their locations so, at a later date when the camera is working, I can go back and photograph them. I compared the drawn pictures to known images of maize phytoliths, and their morphologies are analogous to the two left-hand images in Fig. 16 (see Fig. 17 for a side-by-side comparison). I am confident what I found are maize phytoliths.

Discussion

After the experiment was completed, Dr. Scott provided me with additional information on the dental calculus. The table from *Methods* is reproduced below to include my findings and which dental calculus was of pre- and post-Medieval age.

Sample Number	Pre- or Post-Medieval	Maize Phytoliths?
15-20	Assumed PM	Three
17-53	Medieval	None found
17-68	Medieval	None found
17-70	Medieval	None found
17-77	Medieval	None found
17-86	Medieval	None found
17-103	Medieval	None found
19-7	Assumed PM	None found
19-15	Assumed PM	None found
19-17	Assumed PM	None found
19-23	Assumed PM	None found
29-4	Assumed PM	None found
29-27	Assumed PM	None found
29-48	Assumed PM	None found
29-67	Assumed PM	None found
29-159	Assumed PM	None found
62-71	Unknown	None found

I was excited to find sample 15-20 was potentially post-Medieval: this proved the problems often associated with using phytoliths to reconstruct paleodiet (that extraction, isolation, and identification are all time-consuming and complex) can be overcome with proper equipment and a commitment to their analysis.

I was disappointed, however, that maize phytoliths were not found in the other nine potentially post-Medieval samples. This disappointment has led me to consider what the problems were with this experiment and how the methodology can be improved:

1. I would spend more time examining each slide. While examining the slides, I often got frustrated, tired, and distracted and gave up looking. This is probably the main reason no maize phytoliths were found on the post-Medieval slides. As a change, instead of only searching the slides for 30 minutes, I would allot myself one hour per slide and shut the door to the physical anthropology laboratory to avoid distractions.
2. I would use a scanning electron microscope in addition to the darkfield microscope. This microscope is more powerful than the darkfield and gives closer views of particles on slides. In all the journal articles I read, a scanning electron microscope was used to search dental calculus and dental enamel for phytoliths.
3. I would stain the slides with dye in an attempt to make any particles or phytoliths stand out more. Because phytoliths are miniscule (20-80 nμ) and difficult to find, this would help me spot and then focus on darker images under the microscope.
4. I would enlist the aid of other researchers to share microscope time in the search for phytoliths. Eyeballing slides is a time consuming task and could be better accomplished by more than a single individual.

5. I would follow the methodology of Fox et al. (1996). Although more chemicals were used and it seemed more complex, this procedure has been shown to work. Although I would not test the stomach soil of the skeletons, the methodologies of the dental calculus and enamel would be followed as closely as possible (perhaps with the exclusion of the gold).

Conclusion

While phytoliths are useful in archaeology and paleoecology, they are better utilized in physical anthropology to reconstruct diets of past human populations. The best and most direct method for doing this is not the commonly used carbon and nitrogen isotope analysis. Rather, the method presented in this thesis (and in Fox et al. [1996)] provides the best evidence of what humans ate. This experiment, and phytolith research in general, has its limitations, though. First, phytoliths can only tell researchers what vegetables people ate. Second, because not all plants produce phytoliths, only those vegetables that do can be discerned in this manner. Third, phytoliths are so small, microscopes have to be adjusted to just the right resolution to be seen and trying to find that resolution can become tedious. In some journal articles, the authors said their microscopes were adjusted to 3000-5000X resolutions when they found phytoliths. The microscopes in the physical anthropology laboratory do not go this small, so when the potential phytoliths were seen, it was difficult to confirm if they were phytoliths. Fourth, and perhaps most importantly, because the methods and principals for recovering phytoliths from dental calculus are not well established at this time, researchers have avoided phytoliths entirely. Although Armitage (1975) found festucoid grass phytoliths in the calculus of cattle from prehistoric and historic sites in Britain, Hillson (1986:302) observes “food debris is only rarely incorporated into most plaque.” Rovner (1988) and Hillson’s (1986) positions have probably discouraged researchers from pursuing this avenue of inquiry, but it has never been properly tested for human dental calculus. There are very few articles about such research so experiments, such as this one, are just feasibility studies.

These limitations can be overcome, though, and when they are, the unexplored potential of human dental calculus for unlocking the mysteries of earlier diets and food dispersal and adoption are enormous. This method can revolutionize paleodietary analysis in bioarchaeology because researchers will, for the first time, be able to directly observe the plants which were ingested by earlier human populations. From there, the spread of different domesticates can be followed throughout the world, following the Age of Discovery, as Old World domesticates go to the New World and New World domesticates go to the Old World. The examination of how different domesticates moved from one region to another following domestication can also be studied. This includes the spread of agriculture in Europe after the movement of Indo-European populations into the Balkans and beyond.

There are several other avenues of research which can be done in conjunction with this experiment. One is to test the abrasiveness of maize phytoliths using the Moh test. If maize phytoliths are deemed abrasive, the enamel of pre- and post-Medieval Spaniards can be looked at for any dental wear. Since the Spaniards’ diets changed so much after Native American domesticates were introduced to their country, there is bound to be some change in their dental patterns. Another potential avenue of research is to see if food processing has anything to do with how phytoliths are shaped. Phytoliths taken directly from soil can be compared to those taken from teeth. This can add to the knowledge Fox et al. (1996) and Rovner (1988) argue is imperative for researchers to have when utilizing this method.

If researchers expend enough time and dedication to find phytoliths, they can be found. My hope is this study has shown others this research is not only possible and interesting, it is potentially a revolutionary method for directly determining the paleodiets of earlier human populations.

Literature Cited

Baer P. 1968. Use of laboratory animals for calculus studies. Ann New York Acad Sci 153:230-239.

Crosby AW. 2004. Ecological imperialism: the biological expansion of Europe, 900-1900. Cambridge: Cambridge University Press.

Danielson DR, Reinhard KJ. 1998. Human dental microwear caused by calcium oxalate phytoliths in prehistoric diet of the Lower Pecos Region, Texas. Am J Phys Anthropol 107:297-304.

Epstein E. 1994. The anomaly of silicon in plant biology. Proc Nat Acad Sci USA 91:11-17.

Fox CL, Juan J, Albert RM. 1996. Phytolith analysis on dental calculus, enamel surface, and burial soil: information about diet and paleoenvironment. Am J Phys Anthropol 101:101-113.

Gugel IL, Grupe G, Kunzelmann KH. 2001. Simulation of dental microwear: characteristic traces by opal phytoliths give clues to ancient human dietary behavior. Am J Phys Anthropol 114:124-138.

Hillson S. 1986. Teeth. Cambridge: Cambridge University Press.

Kaufman PB, Dayanandan P, Franklin CI. 1985. Structure and function of silica bodies in the epidermal system of grass shoats. Ann Bot 55:487-507.

Lieverse AR. 1999. Diet and the aetiology of dental calculus. Int J Osteoarchaeol 9:219-232.

Lu HY, Liu ZX, Wu NQ, Berne S, Saito Y, Liu BZ, Wang L. 2002. Rice domestication and climatic change: phytolith evidence from East China. Boreas 31:378-385.

Ordish G, Hyams E. 1996. The last of the Incas: the rise and fall of an American empire. New York: Barnes & Noble.

Piperno DR. 2001. Phytoliths. Center for Tropical Paleoecology and Archaeology Smithsonian Tropical Research Institute 3.1:235-251.

Piperno DR. 2006. Phytoliths: a comprehensive guide for archaeologists and paleoecologists. Lanham: AltaMira Press.

Pohl MED, Piperno DR, Pope KO, Jones JG.   2007. Microfossil evidence for pre-Columbian maize dispersals in theneotropics from San Andrés, Tabasco, Mexico. Proc Nat Acad Sci USA 16:6870-6875.

Rovner I. 1988. Macro- and micro-ecological reconstruction using plant opal phytolith data from archaeological sediments. Geoarchaeol Int J 3.2:155-163.

Sangster AG, Hodson MJ, Tubb HJ. 2001. Silicon deposition in higher plants. Silicon in Agriculutre 85-113.

Appendix A

Families where production is high, phytoliths specific to family are common, and sub-family and genus-specific forms occur, sometimes widely in the family Monocotyledons: Arecaceae*, Cyperaceae* (*Cyperus/Kyllinga**, *Carex**), Heliconiaceae** (*Heliconia***), Marantaceae** (*Maranta**, *Calathea**), Musaceae* (*Musa***), Orchidaceae, Poaceae* (*Chusquea***, *Streptochaeta***, *Tripsacum**, *Zea**), Zingiberaceae*

Dicotyledons: Acanthaceae (*Mendoncia*), Annonaceae, Burseraceae* (*Bursera**, *Canarium**, *Protium**), Chrysobalanaceae*, Asteraceae, Cucurbitaceae* (*Cucurbita**, *Lagenaria**), Dilleniaceae, Fagaceae, Magnoliaceae (*Magnolia***, *Talauma***), Moraceae* (*Morus**), Podostemaceae, Ulmaceae* (*Celtis**), Urticaceae (*Boehmeria***, *Pilea***).

Pteridophytes: Equisetaceae (*Equisetum***), Hymenophyllaceae (*Trichomanes***), Selaginellaceae

Families where production is not high in many species, but where family and genus-specific forms occur
Pinaceae (*Pinus***, *Pseudotsuga***), Chloranthaceae (*Hedyosmum**), Dipterocarpaceae Euphorbiaceae* (*Sapium**), Flacourtiaceae

Examples of families where phytoliths have not been observed, or where production is generally uncommon and of lesser taxonomic utility
Amaranthaceae, Araceae, Araucariaceae, Bignoneaceae, Cactaceae, Chenopodiaceae, Convolvulaceae, Dioscoreaceae, Lecythidaceae, Liliaceae, Melastomataceae, Myrtaceae, Podocarpaceae, Rubiaceae, Sapindaceae, Solanaceae, Taxodiaceae, Tiliaceae

*Reproductive structures (fruits and seeds) also produce high amounts of diagnostic phytoliths.
*Underground organs (roots, tubers, and corms) contribute high amounts of diagnostic phytoliths.

Figure 1. Patterns of phytolith production (Piperno, 2001:237).

Figure 2. Phytolith from a bottle gourd (Piperno, 2001:240).

Figure 3. Phytolith from a domesticated species of squash (Piperno, 2001:240).

Figure 4. Pleated calculus from sample 17-047 (Photo, G.R. Scott).

Figure 5. Calculus from sample 17-069 (Photo, G.R. Scott).

Figure 6. Subgingival calculus from sample 17-107-13c (Photo, G.R. Scott).

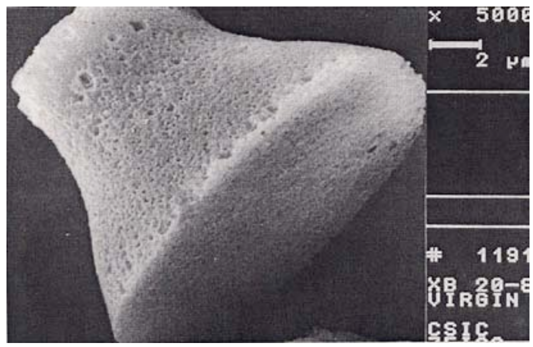

Figure 7. Silica phytoliths from a modern Poaceae plant (Fox et al., 1996:105).

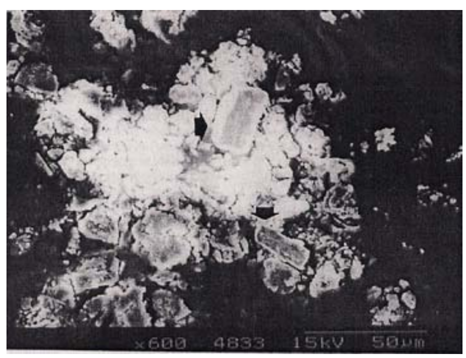

Figure 8. Calculus sample with two phytoliths (arrows are pointing to them). (Fox et al., 1996:111).

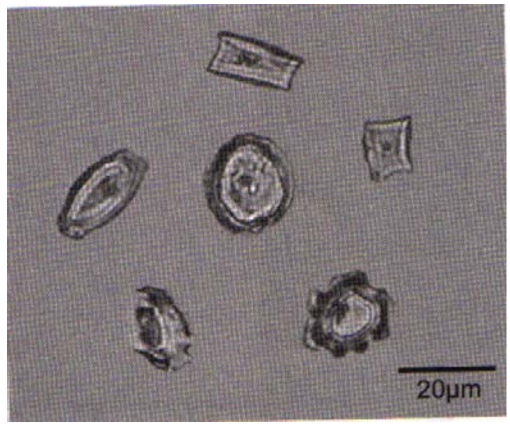

Figure 9. Rondel phytoliths from teosinte (Piperno, 2006:204).

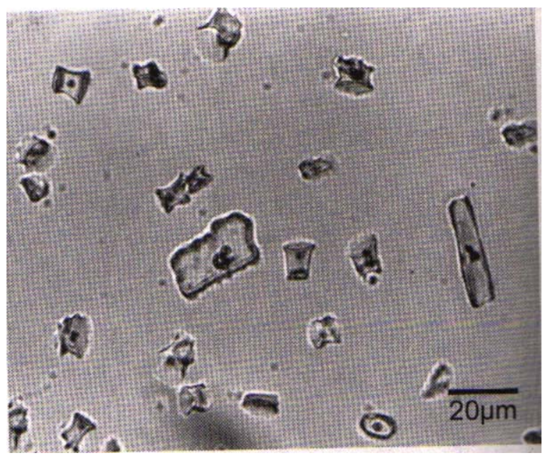

Figure 10. Rondel phytoliths from a maize cob (Piperno, 2006:204).

Figure 11. Bilobate phytoliths characteristic of warm, moist climates (Piperno, 2006:188).

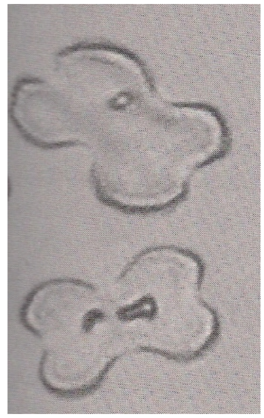

Figure 12. Cross-shaped phytoliths characteristic of warm, moist climates (Piperno, 2006:188).

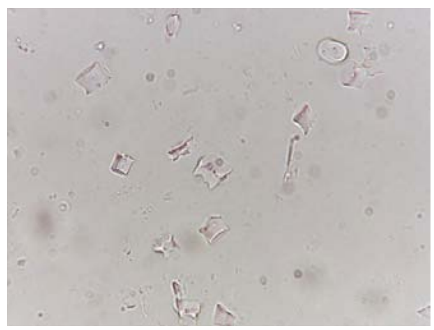

Figure 13. Rondel phytoliths characteristic of cold climates (Pohl et al., 2007).

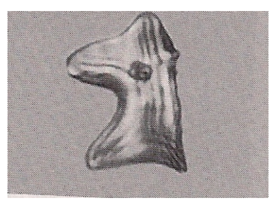

Figure 14. Saddle shaped phytolith characteristic of either a warm, dry climate (short-saddle) or a tropical and subtropical, humid climate (long-saddle) (Piperno, 2006:188).

Figure 15. Cathedral of Santa Maria floor plan (Photo, G.R. Scott).

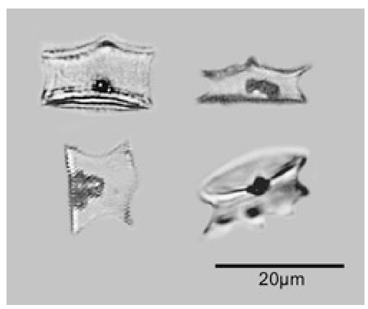

Figure 16. Maize phytoliths (Piperno, 2006:204).

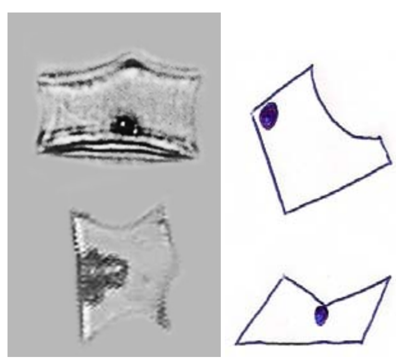

Figure 17. Maize phytoliths (Piperno, 2006:204) and sketches of what the potential maize phytoliths I found look like.